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EFFECT OF EXPOSURE TO LOW TEMPERATURE ON BLOOD CLEARANCE OF CARBON AND BACTERIA IN MICE

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ABSTRACT

Mice housed at 5° C clear carbon from the blood more slowly than animals at 25° C but those in the cold clear at the same rate even after exposures of 2, 18 or 72 hours prior to the test. Bacteria are also "cleared" uniformly at the two temperatures when a highly virulent strain of Salmonella typhimurium is injected intravenously but not when one of low virulence is used. Here, mice at 25° C show a steady decline in bacteremic level but not those at 5° C. The LD_{50} dose of bacteria via the intravenous route is higher than the intraperitoneal dose with strains of each virulence in mice kept at 25° C but it is clearly lower for the mice housed at 5° C. In line with these findings is the observation that exposure to 5° C for 8 hours postinfection followed by 16 hours at 25° C yields mortalities similar to continuous exposure to 25° C and vice versa.

PUBLICATION REVIEW

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SECTION 1. INTRODUCTION

Mice in individual compartments free of bedding and exposed to ambient temperatures of 5° C are significantly more susceptible to experimental infection with low virulent strains (but not those of high virulence) of either Salmonella typhimurium or Staphylococcus aureus compared with mice similarly housed and maintained at 25° C (Previte and Berry, 1962; Miraglia and Berry, in press). Mice in the cold are also more susceptible to injections of bacterial endotoxins than those at usual room temperatures (25° C) (Previte and Berry, 1962, 1963). The basis for these differences is not understood, yet the literature contains reports (Halpern et al, 1951; Taylor and Dyrenforth, 1938) indicating that hypothermia in experimental animals suppresses the cellular defense mechanism as judged by carbon clearance or bacterial clearance, but there are conflicting data (Fedor et al, 1956; Frank et al, 1956). If this were a predominant factor in modifying the response of the cold exposed mice to either infection or endotoxin, it should be readily demonstrable. It is known, of course, that reticuloendothelial "block" (or impairment) sensitizes an animal not only to infectious challenge but to bacterial endotoxin as well.

With these possibilities in mind, the experiments described below were undertaken. Blood clearance of carbon and of both high and low virulent strains of S. typhimurium were determined in mice exposed to 5° C and to 25° C. It was also considered relevant to establish the relationship between duration of exposure to cold and the alteration in host response to infectious challenge.

SECTION 2. METHODS

Carbon Clearance. The method of Benacerraf et al (1959) was modified slightly to comply with the specific requirements of the experiments. The carbon suspension (manufactured by Gunther Wagner, Hannover, Germany,

and designated C11/1431a) contained 10% carbon, 4.3% fish glue, 1% carbolic acid and water. The size of the carbon particles was about 200 to 300 Angstroms. This material diluted 1:4 in pyrogen free isotonic NaCl solution (Baxter Laboratories) was injected intravenously (0.2 ml) in the tail vein of mice. After 10 and 25 minutes, blood from the retroorbital plexus was drawn into a 0.1 ml pipette and transferred to a Coleman cuvette containing 8 ml of 0.1% sodium carbonate. From a standard curve, the amount of carbon remaining in the blood was evaluated following a transmittance reading at 675 mμ in a model 14 Coleman spectrophotometer. The phagocytic index (K) of Benacerraf was calculated for each mouse from the data obtained but was not converted to the corrected value (α) since the weights of livers and spleens were sufficiently uniform to make the calculation equivalent to dividing all K values by a constant.

Bacterial Clearance. Both the highly virulent S. typhimurium strain SR-11 and the less virulent strain RIA were grown in brain-heart infusion broth (Baltimore Biological Laboratories) for an overnight period of 17 hours. The culture was diluted in pyrogen free saline (Baxter) 1:200 such that 0.2 ml contained about 10^6 cells. This volume was injected intravenously and a dilution count was then made on each suspension to make certain of the number of cells administered. Without exception, about 10^6 cells were present.

At intervals postinjection blood was drawn from the retroorbital plexus and dilution counts were made on individual animals. This was considered preferable to sacrificing a mouse for each determination.

Experimental Infections. Both strains of S. typhimurium were grown in brain-heart infusion broth for about 17 hours, diluted with pyrogen free saline and injected either intravenously or intraperitoneally in numbers determined by dilution counts. The mice were housed individually in compartmented cages without bedding but with food and water available at all times. They were kept either continuously at 5° C or at 25° C, or were transferred back and forth at intervals described below.

Temperature Rooms. A walk-in refrigerator maintained at 5° ± 2° C was used for cold exposure. The mice were handled as previously described (Previte and Berry, 1962; Miraglia and Berry, in press). In essence, they were placed out of air drafts, they were kept dry, and a time switch insured 12 hours of light and 12 hours of darkness per day. The 25° ± 2° C room was similarly illuminated and conditions were otherwise as identical as possible for the mice except for temperature.

Mice. CF-1 female mice (Carworth Farms) were used exclusively in these studies. Weekly shipments of animals weighing about 18 g were received. They were housed 10 per cage with pine shavings as bedding in an animal room held at 25° ± 2° C for at least one week. When they weighed 20 ± 1 g, they were then ready for experimental study. They were fed Dietrich and Gambrill's pathogen free mouse food ad libitum and water was available at all times.

SECTION 3. RESULTS

Carbon Clearance. Table 1 contains the K values calculated for individual mice in each of four experimental groups. The mean value for each group is given at the bottom of the table and the significance of differences between means as calculated by the rank order test of White (1952) are also presented. It is apparent that an exposure to 5° C for as little as two hours (Group 2) lowers the ability of the reticuloendothelial system (RES) to clear carbon from the blood compared to that of mice held at 25° C. Maintaining mice at 5° C for 18 hours has no effect on the activity of the RES greater than that seen after two hours. In fact, the mean K values of Groups 2 and 3 are identical.

The latter, compared to that of Group 1, gives a higher level of significance than Group 2, probably because of the larger sample size. Mice kept at 5° C for 72 hours show the lowest average K value (Group 4) of any of those studied. This Group compared to Group 1 is statistically the most significantly depressed in RES activity but it is not significantly lower by rank test than the other two groups exposed to 5° C. On the basis of these findings, it seems perfectly clear that cold exposure suppresses carbon clearance in mice.

Clearance of Bacteria. The results presented in Table II reveal additional relationships of some interest. In the first place, bacteria, in contrast to carbon particles, are not completely removed from the blood after an interval as long as 24 hours. This may obviously result as much from reinfection of the circulation as from failure of phagocytic cells to ingest the microorganisms. The finding of a persistent bacteremia following intravenous infection is not unexpected, however, in light of the detailed studies reported by Rogers and his associates (Rogers, 1956; Rogers and Melly, 1957). Apparently one must anticipate with certain pathogens a more or less steady state between the rate of ingestion and rate of blood re-entry.

TABLE I

The effect of low temperature exposure on carbon clearance in mice. Each animal was injected intravenously with 0.2 ml of a suspension containing 5 mg of carbon. Blood samples were taken at 10 and 15 minutes postinjection and each value presented in the Table was calculated from the formula $K = \log C_{10\ min} - \log C_{25\ min} / T$, where T = time in minutes between the two carbon determinations.

K Values for Mice Subjected to				
25° C Control Group 1	2 Hours Exposure to 5° C Before Injection Group 2	18 Hours Exposure to 5° C Before Injection Group 3		72 Hours Exposure to 5° C Before Injection Group 4
.027	.017	.018	.026	.020
.033	.022	.023	.017	.016
.019	.020	.025	.017	.021
.036	.022	.034	.027	.013
.041	.021	.018	.007	.015
.023	.031	.028	.011	.013
.030	.019	.030	.032	.020
.048	.014	.018		.021
.035	.035	.028		.019
Mean .032	.022	.022		.018
	P = 0.05 for Groups 1 and 2	P = 0.01 for Groups 1 and 3		P = 0.001 for Groups 1 and 4

TABLE II

Number of S. typhimurium, strain SR-11 and strain RIA, per ml of blood remaining at specified times following intravenous injection of about 10^6 cells at time zero in mice maintained at either 5° C or 25° C. The values presented are the mean of the number of separate determinations shown in parentheses. Significance of difference between means calculated by rank test.

Time Post-Injection (Hours)	Number of SR-11 Remaining in Mice Housed at		Number of RIA Remaining in Mice Housed at		Significance of Temperature Difference for	
	5° C	25° C	5° C	25° C	SR-11	RIA
3			6.1×10^3 (9)	1.9×10^3 (11)		<0.01
5	1.5×10^3 (13)	1.0×10^3 (12)	3.1×10^3 (13)	4.0×10^2 (18)	N.S*	<0.001
7	1.4×10^3 (14)	1.4×10^3 (11)	1.2×10^3 (13)	2.4×10^2 (14)	N.S	<0.001
24	1.7×10^3 (7)	1.5×10^3 (9)	1.0×10^3 (3)	3.8×10^1 (5)	N.S	<0.001

*N.S. = not significant.

This is strikingly clear with the virulent SR-11 strain where in mice at each temperature a constant bacteremic level exists from five hours postinfection until at least 24 hours. One should anticipate, on the basis of prior studies, persistence of this level until about 12 to 24 hours before death when a logarithmic increase ensues (Berry and Mitchell, 1954).

A second interesting finding is the behavior of RIA compared to that of SR-11. The strain of low virulence becomes progressively fewer in number in the blood of mice housed at 25° C but shows essentially a constant number in mice at 5° C. Possibly this is no more than another way of saying that mice at 5° C are more susceptible to RIA than mice at 25° C. Regardless of interpretation, the findings are clear. Temperature has no measurable effect on the bacteremic level of mice infected intravenously by a highly virulent strain of S. typhimurium, while exposure to low environmental temperature (compared to exposure to room temperature) increases bacteremia in mice infected with an organism of low virulence.

Temperature Effect on LD_{50} Dose of S. Typhimurium Ingested by Different Routes. Several important relationships are contained in the data summarized in Table III. Note first that infection by either the intraperitoneal (i.p.) or the intravenous (i.v.) routes with the virulent SR-11 strain of S. typhimurium is influenced only slightly by temperature. The increase in LD_{50} dose at 25° C from 7 to 100 cells (for i.p. vs. i.v., respectively) is to be expected for enteric forms. Schewe (1958) recently reported the need for approximately a one log increase in number of S. typhimurium required to kill mice in a given time period by i.v. inoculation in comparison with i.p. injections. At 5° C, however, the number required is too small by either route to make an effect detectable.

Interesting results were obtained, however, with the low virulent RIA strain. Temperature makes a two log difference in LD_{50} following i.p. infection but a four log difference in animals infected via the i.v. route. There is nearly a one log decrease at 5° C in LD_{50} dose i.v. versus i.p. The reverse change was seen, on the other hand, at 25° C. This latter effect appears to be independent of the virulence of the organism used at normal room temperatures but with the low virulent form an effect is seen that is not evident with SR-11. It would seem, therefore, that suppression of the RE system of mice by temperature (cf. Table II) makes infectious challenge more lethal i.v. than i.p., possibly because of the relative accessibility of the pathogens to phagocytic cells by the different pathways of administration. Much work remains to make this more than an hypothesis.

TABLE III

The LD_{50} dose of two strains of S. typhimurium for CF-1 mice kept continuously at two environmental temperatures for the duration of the experiment, 14 days. Each value is based on results obtained with at least fifty animals. Two routes of infection are compared.

Route of Infection	LD_{50} Dose of S. typhimurium of Strain SR-11 Given to Mice Housed at		RIA Given to Mice Housed at	
	5° C	25° C	5° C	25° C
Intraperitoneally	7 cells	7 cells	3.8×10^3 cells	4.1×10^5 cells
Intravenously	<10 cells	10^2 cells	5.4×10^2 cells	7.8×10^6 cells

Effect of Discontinuous Exposure to Cold on Susceptibility to Infection. Four groups of mice were infected intraperitoneally with an LD_{50} dose of S. typhimurium, strain RIA. This is 3.8×10^3 cells. Group I was exposed continuously postinfection to 5° C. Group 2 was placed at 5° C for the first eight hours postinfection and then at 25° C for the remaining 16 hours of the day. Each day thereafter for a period of 14 days, at which time the experiment was terminated, the same cycle of exposure to 5° C and to 25° C was repeated. Group 3 was exposed to temperatures in the reverse order of Group 2; i.e. the first eight hours postinfection were at 25° C and the subsequent 16 hours were spent at 5° C. This cycle was also repeated for 14 days. Group 4 was similar to Group 1 except that an exposure to 5° C for 18 hours occurred prior to infection. The results are presented in Table IV. Only Group 2 is significantly different from the other groups, and specifically Group 1. It seems clear that the outcome of this particular infection is resolved during the 16-hour period following the initial eight hours postinfection. Mice infected with this dose of RIA survive completely when kept continuously at 25° C. Thus, Group 2 comes close to behaving in this way while Group 3 is more like Group 1 than Group 2. Apparently, therefore, the interaction between host and parasite is not resolved in all circumstances during the initial stages of disease as has been reported in the literature (Miles, 1956).

SECTION 4. DISCUSSION

Exposure of mice to a low environmental temperature of 5° C under the conditions of the experiments described above leads to a transitory hypothermia of between 1° and 2° C during the first four to five hours but by 18 hours normothermia is usually observed (Previte and Berry, 1962). In the measurements of carbon clearance, Group 2 mice, those held at 5° C for two hours before testing, are hypothermic but those of Group 3, exposed to 5° C for 18 hours at the time of the determinations, are normothermic. The mean values for K are identical in the two groups. One must assume, therefore, that differences in body temperature of the magnitude involved contribute in no major manner to the efficiency of carbon clearance from the blood stream. Of greater significance is the probable effect of adrenocortical secretions which are known to suppress the activity of the RE system (Germuth, 1956). One could simulate, perhaps, the effect of cold exposure by an injection of cortisone.

TABLE IV

A comparison of the effect of continuous exposure versus intermittent exposure to 5° C on susceptibility to infection with the low virulent strain of S. typhimurium, strain RIA. All animals were infected intraperitoneally and treated as shown. The period of observation was 14 days.

Experimental Treatment	Number Surviving / Number infected	Probability* Compared to Group 1
Group 1. Continuous exposure to 5° C postinfection.	9/20	
Group 2. First 8 hours post-infection at 5° C then 16 hours at 25° C. Cycle repeated for 14 days	18/20	.014
Group 3. First 8 hours post-infection at 25° C then 16 hours at 5° C. Cycle repeated for 14 days.	13/20	N. S. **
Group 4. 18 hours preinfection exposure to 5° C and continuous at 5° C postinfection.	7/20	N. S.

* Calculated by method of Wilcoxon (1958).

** N. S. = not significant.

The contrast in behavior of the high and low virulent strains of S. typhimurium is most interesting. A relatively constant level of bacteremia during the first 24 hours postinfection occurred with SR-11 (highly virulent strain) and also with RIA in mice at 5° C. RIA in mice at 25° C progressively declined in number. These last were the only mice able to survive the infection while those in all other treatment situations succumbed. The persistent bacteremia under the specific conditions is associated with a prognosis of death. The level of the bacteremia observed in mice that were to die deserves comment. The blood volume in mice of the size employed is no greater than 3 ml (Berry and Smythe, 1961). Thus, about 3×10^5 organisms per ml of blood were injected but the steady-state level is 1% or less of that amount. Most bacteria, therefore, are cleared and the mouse is able to keep the number low in comparison with the size of the challenge. Apparently it is more the ability to prevent reinvasion of the blood by the pathogens than the capacity for ingesting them initially that determines the outcome of the infection. This hypothesis is borne out by the remainder of the work described in this report.

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